CUTANEOUS FUNGAL PATHOGENS IN PATIENTS AT THE HOSPITAL OF HUE UNIVERSITY OF MEDICINE AND PHARMACY, VIETNAM

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Abstract

Background: The superficial cutaneous mycoses are usually confined to the outer layers of skin, hair, and nails, and do not invade living tissues. Dermatophytes and yeasts are the main pathogenic agents of these mycoses. In addition, some molds are mentioned as causative agents of these diseases. This study aimed to identify the cutaneous fungal species from 181 dermatophytosis patients in Hospital of Hue University of Medicine and Pharmacy, Vietnam. **Methods:** A cross sectional descriptive study was carried on 181 dermatophytosis patients with positive direct examination of fungal infection. These specimens were isolated on selective media for fungal etiologies. Identification of fungal etiologies were performed by morphology and certain biochemical reactions. **Results:** *Dermatophytes* were the most prevalent cutaneous fungal infection (90.6%), followed by yeasts (7.7%) and then *non dermatophytes molds* (1.7%). As the causative dermatophytes species, *Trichophyton rubrum* was the most frequently isolated pathogen (58.0%). *T.rubrum* and *T.mentagrophytes* were isolated from all the dermatophytosis clinical types. *Candida sp* and *Trichosporon cutaneum* were etiological agents of paronychia - onychomycosis. *Fusarium sp.* was pathogenic in onychomycosis and tinea corporis. *Scopulariopsis sp.* caused of onychomycosis. **Conclusion**: The highest rate of cutaneous fungal pathogens was anthropophilic *dermatophytes,* and followed by zoophilic dermatophytes. *Candida albicans* and *Candida non albicans* were found in paronychia - onychomycosis patients. In addition some nondermatophyte mold infections were found in both of skin and nail.

Key words: *Dermatophytosis, molds, dermatophytes, Candida sp.*

1. INTRODUCTION

The cutaneous mycoses are superficial fungal infections of the skin, hair or nails. Many studies performed all over the world have demonstrated that most cases of these diseases are caused by dermatophytes [2, 4,18, 20]. However, other studies have recorded that yeasts and non dermatophyte molds may play a role, particularly in onychomycosis [3, 4,18,].

Dermatophytes, a group of fungi have the capacity to invade keratinized tissue, so that they are able to invade the hair, skin, and nails of living host. There are 55 dermatophyte species divided into three genera: *Trichophyton, Microsporum, and Epidermophyton*. Relating to human pathogens are 23 *Trichophyton* species, 15 *Microsporum* species and one *Epidermophyton* species (*E.floccosum*) [20]. These species are classified as anthropophilic, geophilic or zoophilic dermatophytes according to their habitat [20]. Some anthropophilic dermatophytes are very popular all over the world such as *T.rubrum, T.tonsurans*, *T.mentagrophytes*, *T.violaceum*, *M.audouinii, M.ferrugineum, E.floccosum*, *M.canis, M.equinum, T.verrucosum* are the main zoophilic dermatophyte. *M.*

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gypseum, M. praecox, T. ajelloi are belonging to geophilic group [1]. Dermatophyte infections are divided according to the affected body sites into tinea capitis (scalp), tinea pedis (foot), tinea corporis (body), tinea cruris (groin), tinea manuum (hand), and tinea unguium (nail) [20]. Dermatophyte infections are common in both developed and developing countries. The fungal species distribution depends on geographic region. Not all species of dermatophytes are popular in over the world. Some fungal species have been isolated from every continent, others have geographically limited in some countries or areas. The distribution of dermatophytes varies from region to region and over time, tends to influence by some factors, such as age, socio-economic factors, lifestyle, close contact to pets, and climate [7, 8].

Candida sp, the component of the normal human microbiota flora, is considered opportunistic yeast. It could affect mucous membranes, skin and nails. This fungus has a worldwide distribution and affects people of all ages. In the other hand, *Trichosporon sp., Geotrichum sp*, and other yeasts are mentioned as a yeast causative agent in human [10]. Today, several reasons have been suggested to explain the increased incidence of yeast infections, including the overuse of antibiotics, receiving aggressive regimens of chemotherapy in cancer, or taking immunosuppressive drugs for rheumatologic or autoimmune diseases, as well as the increasing number of immumocompromised patients.

Molds rarely cause cutaneous infections. Occasionally, secondary skin infections can occur in immunosuppressed patients, such as with leukemia and after stem cell transplantation. Primary skin infections due to molds are even rarer. Nowadays, non dermatophyte molds like *Fusarium sp., Acremodium sp*... are considered emerging pathogens in onychomycosis [3, 9].

Vietnam is a tropical country. Hue city is located in central of Viet Nam, where heat and humidity are good conditions for cutaneous fungal growth. It is suitable for high rate of dermatophytosis. Our previous study in 2003, the rate of dermatophytosis was 52% in the Hospital of Hue Medicine and Pharmacy University. The etiological agents of dermatophytosis have been well studied in many countries, but there was not any data in Vietnam as well as in Hue city.

This study aimed to identify the fungal cutaneous pathogens from March 2010 to March 2011 in Parasitology Department of Hue University of Medicine and Pharmacy Hospital, Vietnam.

2. MATERIALS AND METHODS

Cross-sectional descriptive study was carried out on Hue Medicine and Pharmacy Hospital, Vietnam. A total of 181 patients with clinical cutaneous fungal infections attended the Parasitology Department for laboratory diagnostic from March 2010 to March 2011.

2.1. Sampling

The skin scraping and hair specimens were collected from these patients by scraping by the edge of the lesions using the clean slide and Scotch test technique. Nail specimens were clipped with scissors and the debris under the nail plate were collected using a sterile scalpel. The hair samples were placed in clean sterile Petri dishes for direct examination with KOH 20% solution and cultivation.

We also collected the data regarding demographical data, date of sample collection, site of the lesions following the classified clinical form of infection by ICD-10. Clinical forms were divided in eight types, including tinea pedis, onychomycosis, tinea corporis, tinea capitis, tinea manuum and intertrigo, tinea cruris, paronychia, and multiplex clinical type (more than one body infected sites).

2.2. Mycological examination

All the specimens were firstly subjected to direct microscopic examination by using 20% KOH solution for the presence of fungal hyphae, spore or yeast. Then the filamentous fungi positive samples in direct examination were cultured on the Sabouraud's dextrose agar supplemented with chloramphenicol (50mg/l) and cycloheximide (0.5mg/l) for isolation and identification of

dermatophyte by macroscopic and microscopic morphology of the colonies. We also cultivated these samples on the Sabouraud's dextrose agar supplemented with chloramphenicol (50mg/l) without cycloheximide (0.5mg/l) for isolation and identification of non dermatophyte mold and yeast. Addition cultural on Corn Meal agar medium following Dalmau technique and Auxacolor test were used to identify yeast species. The identification of filamentous fungi was based on the observation and description of the macroscopic and microscopic characteristics of the colonies obtained in the culture.

The statistical analysis was performed using the software SPSS version 11.5.

3. RESULT

A total of 181 patients having superficial mycosis features and positive fungal direct examination were included, with 71.3 % males (129/181) and 28.7% females (52/181). The mean age of patient was 24 years old. The youngest patient was 4 years old and oldest was 74 years old. The distribution of dermatophytosis isolated in relation to different age groups is shown in Table 1. Analyzing the number of cases per age group, patients aged 16 - 25 years accounted for most cases (n= 136;75.1%), followed by patients aged 26 - 35 years (n= 14, 7.7%) and patients aged 46 - 65 years (n=13, 7.2%). However, there were not more patients in each age group in our study, with the exception of the group 16 -25 years, so it was difficult to consider the difference in frequency of dermatophytosis according to age groups.

The most frequent agent isolated from culture was dermatophyte (90.6%), followed by yeast (7.7%) and non dermatophyte mold (1.7%). The detailed species identification was shown in Table 2. In three genera of dermatophytes, the most frequency was *Trichophyton sp* (82.9%), followed *Microsporum sp* (7.2%) and *Epidermophyton floccosum* (0.6%). Regarding in *Trichophyton species*, *T. rubrum* was the most specie isolated (58.0%) and some agents also found such as *T.mentagrophytes*, *T.tonsurans*, *T.violaceum*, *T.erinacei*, *T.schoenleinii*, *T. soudanense*, *T.verrucosum*. In case positive for non dermatophyte mold, three pathogenic species were recognized including *Fusarium onysix*, *Fusarium solani* and *Scopurialopsis sp*.

The highest clinical form was tinea cruris and the incidences of the other clinical types were presented in Table 3.

The dermatophyte species most commonly isolated were *T. rubrum*, followed by *T. mentagrophytes,* in all of clinical forms, except for paronychia. In contract, *Candida sp* and *Trichosporon cutaneum* were the causative agent of paronychia. The distribution of fungal causative agents according to clinical types was presented in Table 4.

Table 1. Distribution of dermatophytosis according to age and gender

Age	Male		Female		Total	
	N	(%)	N	(%)	N	(%)
< 15	5	3.9	2	3.9	7	3.9
16 – 25	105	81.4	31	59.6	136	75.1
26 – 35	10	7.8	4	7.7	14	7.7
36 – 45	3	2.3	7	13.5	10	5.5
46 – 65	6	4.6	7	13.5	13	7.2
> 66	0	0.0	1	1.9	1	0.6
Total	**129**	**100**	**52**	**100**	**181**	**100**

Table 2. The species identification of cutaneous fungal agents

Agents		Number	Percentage (%)
Dermatophytes	*T.rubrum*	105	58.0
	T. mentagrophytes	26	14.4
	T.tonsurans	6	3.4
	T.violaceum	5	2.8
	T. erinacei	3	1.7
	T.schoenleini	2	1.1
	T.soudanense	1	0.6
	T.verrucosum	2	1.1
	M.gypseum	8	4.4
	M.canis	4	2.2
	M.persicolor	1	0.6
	Epidermophyton floccosum	1	0.6
Nondermatophytes molds	*Fusarium solani*	1	0.6
	Fusarium onysix	1	0.6
	Scopulariopsis sp.	1	0.6
Yeast	*Candida albicans*	7	3.9
	Candida parapsilopsis	2	1.1
	Candida tropicalis	1	0.6
	Candida famata	1	0.6
	Candida guilliermondii	1	0.6
	Trichosporon cutaneum	2	1.1
Total		**181**	100

Table 3. Distribution of cutaneous clinical features

Clinical types	Number	Percentage (%)
Tinea capitis	3	1.7
Onychomycosis	7	3.9
Tinea manuum and intetrigo	6	3.3
Tinea pedís	11	6.1
Tinea corporis	52	28.7
Tinea cruris	63	34.8
Multiplex types	28	15.5
Paronychia	11	6.1
Total	181	100

Table 4. Causative agents according to clinical presentations

Clinical feature / Fungal Species	Tenia capitis	Onycho-mycosis	Tinea manuum and intertrigo	Tinea pedis	Tinea corporis	Tinea cruris	Multiple clinical type	Paronychia	Total
T.rubrum	1	4	1	7	30	38	24	0	105
T. mentagrophytes	1	0	1	1	9	11	3	0	26
T.tonsurans	0	0	0	0	1	5	0	0	6
T.violaceum	0	0	0	1	1	2	1	0	5
T. erinacei	0	0	0	0	3	0	0	0	3
T.schoenleini	0	1	0	0	0	1	0	0	2
T.soudanense	0	0	0	1	0	0	0	0	1
T.verrucosum	0	0	0	0	1	1	0	0	2
M.gypseum	0	0	1	0	5	2	0	0	8
M.canis	1	0	0	0	1	2	0	0	4
M.persicolor	0	0	0	1	0	0	0	0	1
E. floccosum	0	0	0	0	0	1	0	0	1
Fusarium solani	0	1	0	0	0	0	0	0	1
Fusarium onysix	0	0	0	0	1	0	0	0	1
Scopulariopsis sp.	0	1	0	0	0	0	0	0	1
Candida albicans	0	0	3	0	0	0	0	4	7
Candida parapsilopsis	0	0	0	0	0	0	0	2	2
Candida tropicalis	0	0	0	0	0	0	0	1	1
Candida famata	0	0	0	0	0	0	0	1	1
Candida guilliermondii	0	0	0	0	0	0	0	1	1
Trichosporon cutaneum	0	0	0	0	0	0	0	2	2
Total	3	7	6	11	52	63	28	11	181

4. DISCUSSION

Cutaneous fungal disease remains a popular clinical condition in humans worldwide. Factors such as age, weather conditions, social practices, and hygiene practices certainly contribute to the epidemiological variations in this disease. In the present study, dermatophytosis affected to male (71.3%) more than females (28.7%). This result was similar to the study of Nishimoto Katsutaro in Japan (2002) [13], and Flores Juan Medina in Peru (2009) [8]. This difference could be explained by the capacity of more body action in men. It related to increase body's sweat, so it is a better factor for fungal growth [19,20]. Althought the mean age of our patients was 24 years old, our study showed the range in age was between 4 and 74 years old. This result revealed that fungal cutaneous infections were common in all of age. Some studies showed that the prevalence and clinical features of dermatophyte were dependent on the age [4,13]. In addition, the study of Pires CA et al in Brazil (2012) showed that the female in age group of 51 to 60 years were the most affected by cutaneous mycoses [14]. However, the distribution of cutaneous mycose was focused on the age group from 16-25 years old in both genders in this study. In order to understand more detailed the influence of age and other affecting factors of this disease, it

should carry out more study about this subject in Vietnam.

The Table 2 showed that the most of fungal cutaneous agents were the septae hypha fungi 92.3% (*dermatophytes* 90.6% and *nondermatophytes molds* 1.7%), the yeast was 7.7%. The predominance of dermatophyte in our study was the similar result of the data reports in etiologies of superficial fungal diseases from many regions on over the world [1]. Howerver, the *nondermatophytes molds* causing disease should be concentrated on in onychomycosis, especially onychomycosis in diabetes mellitus, peripheral vascular disease and activities related to foot trauma patients [3,17,19,20].

In dermatophyte agents, the predominent rate of *Trichophyton sp.* was 82.9%, then *Microsporum sp.* 7.2%, and the lowest rate was *Epidermophyton* 0.6%. In the genus *Trichophyton*, the highest rate was *T.rubrum* (58.0%) and then *T.mentagrophytes* (14.4%). This result was similar the data from other studies in Asia region such as Thailand, India, Srilanka... [1]. The report of Flores Juan Medina et al in Peru showed that the dominent rate of *T.rubrum* was 59.7% and *T.mentagrophytes* was 9.7% to compare with other species [8]. The study of Nishimoto Katsutaro in Japan showed the same result of common rate of *T.rubrum*, then *T.mentagrophytes* [13]. In contract, the study of Bassiri-Jahromi S in Iran showed that the highest percentage of *Epidermophyton floccosum* and then *T.rubrum*, *T.mentagrophytes* [2]. Furthermore, we found that most of dermatophyte agents were anthropophilic fungi (84.7%). In contrast, there were only 10 cases (5.5%) of zoophilic dermatophyte including 3 cases of *T. erinacei,* 2 cases of *T. verrucosum*, 4 cases of *M. canis* and 1 case of *M. persicolor*. Regarding to cases of *T. erinacei* and *T. verrucosum,* we found them from famers. The animal contacting could be risk factors of fungal infection in this case, especially contacting with hedgehog [15]. *T. erinacei* is the rare species of *Dermatophytes.* There were some reported cases of *T. erinacei* infections in Asia, South America and Europe [5,11,16]. Here, we reported three cases of *T.erinacei* in Vietnam.

In addition, there was 1.7% of *nondermatophytic* causative agents with *Fusarium solani, Fusarium onysix* and *Scopulariopsis* in our research. Certain other studies showed that the low percentage of *nondermatophyte molds* but there was diversity of agents such as: *Aspergillus sp.(A.flavus, A.fumigatus, A.terreus, A.niger), Fusarium sp., Acremonium sp*., *Scopulariopsis sp., Cladosporium sp., Trichoderma sp*.[2,9,]. Furthermore, the nondermatophytic agents were often reported in immunocompromised patients such as diabete, especially caused by onychomycosis [12]. However, in our data, *Fusarium onysix* caused tinea corporis, while *Fusarium solani* and *Scopulariopsis* were found with onychomycosis, these infections were found in immunocompetent patients.

The others fungal pathgenic of onychomycosis were *Candida* and *Trichosporon*. The result in Table 2 showed that the prevalence of *Candida sp* was higher than *Trichosporon* (6.6% vs 1.1%). This result was similar Bassiri-Jahromi S, Khaksari AA's study [2].

We classified the clinical types according to CD-10. The Table 3 showed that the high rate were tinea cruris (34.8%) and tinea corporis (28.7%), in contract, the lowst rate was tinea capitis (1.7%).

The result on Table 4 showed that *T.rubrum* and *T.mentagrophytes* were the most common on almost of clinical features except for paronychia. In contract, *Candida sp* and *Trichosporon cutaneum* often caused paronychia and onychomycosis. The rare cases tenia capitis caused by *T.rubrum* (1 case), *T.mentagrophytes* (1 case) and *M.canis* (1 case). The studies of Bassiri-Jahromi S and Nishimoto Katsutaro also indicated that *M.canis* and *T.tonsurans* were the commonly agent of tinea capitis [2,13]. We found three agents cause of onychomycosis including *T.rubrum, T. shoenleini* and *Fusarium solani*. Meanwhile, paronychia was caused by *Trichophyton sp.* and *C.albicans*. Even though *C. albicans* was the most common caused in paronychia, we also found some *C.non albicans* species such as: *Candida parapsilopsis, Candida tropicalis, Candida famata, Candida guilliermondii* and *Trichosporon cutaneum*. In contract, tenia pedis

were caused by *Trichophyton sp.* và *M.persicolor.* Considering fungal species identification of tenia corporis, two dermatophytes g were isolated including *Trichophyton sp, Microsporum sp,* and one species of nondermatophyte - *Fusarium onysix*. The most common causative agents of tenia crusis were *Trichophyton sp, Microsporum sp* and *Epidermophyton floccosum*. Our study was the first study in Vietnam about etiology of dermatophytose and the results were similar to those of certain other studies [2,13].

This study showed that the most fungal causative agents of skin lesions were anthropophilic dermatophyte, and onychomycosis were *Candida sp.* However, certain diversities of other agents comprising *nondermatophyte* and *Candida non albicans* should be noted.

5. CONCLUSIONS

The most common fungal isolates from cutaneous fungal diseases in Hospital of Hue University of Medicine and Pharmacy were *dermatophytes (*90.6%), in which anthropophilic fungi were 84.7%, and zoophilic dermatophytes were in lower rates including: *T. erinacei, T. verrucosum*, *M. canis* and *M. persicolor.* Yeast were often isolated from paronychia and onychomycosis, these species included C. albicans, *C.parapsilopsis, C.tropicalis, C.famata, C.guilliermondii.* In addition, nondermatophyte molds including *Trichosporon cutaneum, Fusarium solani, Fusarium onysix* and *Scopulariopsis* were causative agents in both of skin and nail.

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